Express Mail Label No. Dated: _____

Docket No.: 03818/100L650-US1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Mladen Mercep et al.

Application No.: 10/615,716

Filed: July 8, 2003

Art Unit: 1623

For: COMPOUNDS, COMPOSITIONS AND METHODS FOR TREATMENT OF INFLAMMATORY DISEASES AND CONDITIONS

Examiner: E. Peselev

DECLARATION OF LINDA TOMAŠKOVIĆ UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Linda Tomašković, declare that:

(1) I am a citizen of Croatia and reside in Zagreb, Croatia.

(2) I am currently employed at Pliva-Istrazivacki Institut d.o.o.

(3) I am one of the named inventors of the above-identified application published as U.S. Pat. Pub. No. 2004/0014685

(4) I re-affirm my duty of candor and good faith in dealing with the United States Patent and Trademark Office ("USPTO"), including the duty to disclose to the USPTO all information known to be material to the patentability of the invention as defined in 37 CFR §1.56.

(5) I submit this declaration to support patentability of claims of the present application that have been rejected based on Burnet (US 2004/0087517) on the grounds of either anticipation (prior identical disclosure of the invention) or obviousness.

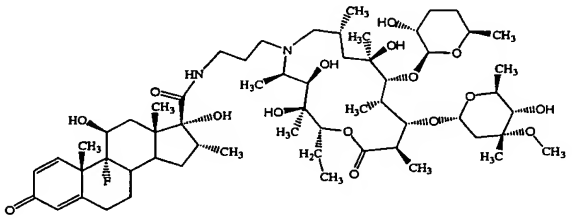
(6) More specifically, I submit this declaration to show that my co-inventors and I conceived and reduced the present invention to practice at a time prior to February 15, 2002, the earliest filing date asserted by Burnet.

(7) From a time considerably earlier than February 15, 2002, my co-inventors and I formed part of a team that devoted considerable resources to an on-going program of development of macrolide conjugates that include an anti-inflammatory moiety linked to a macrolide molecule and that possess anti-inflammatory activity. The macrolides were predominantly azithromycin and other homoerythromycin derivatives.

(8) As evidence of the existence and purpose of this program, and the existence of a broad concept that led to the present invention, I attach a copy of the specification of International Patent Application WO 02/055531 (Exhibit A). Three of my present co-inventors and I are named as inventors in WO 02/055531. This application was filed January 3, 2002. In this document, we broadly state that conjugates having the general formula MLA have anti-inflammatory activity. M represents a macrolide subunit (Formulas M1, through M6), L is a linker linking M and A, and "A represents an anti-inflammatory subunit that can be steroid or nonsteroid." (Exhibit A, paragraph bridging pages 3 and 4, page 3 lines 12-13, pages 4 - 9 (macrolides), and pages 9 -12 (steroids)). Exhibit A also discloses a number of specific macrolide-steroid conjugates (Exhibit A, Table 1, pages 39-43) and their anti-inflammatory activity (Exhibit A, page 3 and pages 25 – 29.

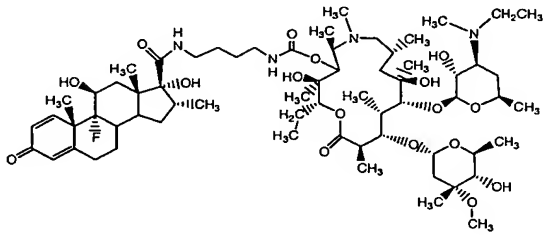
(9) Further, as evidence that my co-inventors and I reduced the present invention to practice prior to February 15, 2002 I submit pertinent excerpts from the laboratory notebook maintained in my laboratory pertaining to some of the compounds that were synthesized early. These entries were made prior to February 15, 2002.

(10) To show that, for example, Compound 1 of the present application was synthesized before February 15, 2002, I submit as Exhibit B pages 16-17, from Laboratory Notebook identified by code LTO0959016A Lab.diary 000959, and an English translation of these pages. Exhibit B sets forth the synthetic protocol for the conjugation of the macrolide and dexamethasone which produced the conjugate designated as Compound 1 in the present application:



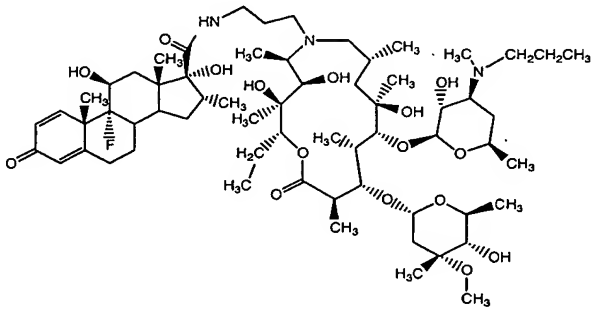
The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also set forth on pages 16 - 17. Additionally, the synthesis of the macrolide-linker and the dexamethasone derivative used to form the conjugate compound 1 described above are provided with amount and identity of reagents used, solvents, and reaction yield in Exhibit C (pages 106-7 of Lab diary 0560,pages 98-99 of Lab diary 0560, and page 59 of Lab diary 0560). These records demonstrate that Compound 1 disclosed in the present application was actually reduced to practice prior to February 15, 2002.

(11) To show that, for example, Compound 4 of the present application was synthesized before February 15, 2002, I submit as Exhibit D pages 77-78 from a Laboratory Notebook identified by code VPO-1131- 077-A Lab.diary 1131, and an English translation of these pages. Exhibit D sets forth the synthetic protocol for the conjugation of the macrolide and dexamethasone which produced the conjugate designated as Compound 4 in the present application:



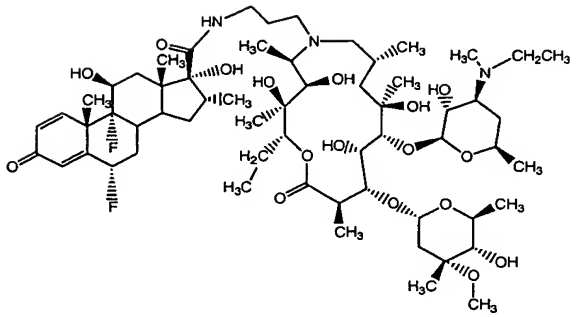
The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. This record demonstrates that Compound 4 within the present application was actually reduced to practice prior to February 15, 2002.

(12) To show that, for example, Compound 9 of the present application was synthesized before February 15, 2002, I submit as Exhibit E page 124 from a Laboratory Notebook identified by code OMA-000961-124-A Lab.diary 000961 and page 22 OMA-001127-022-A Lab.diary 001127, and an English translation of these pages. Exhibit E sets forth the synthetic protocol for the conjugation of the macrolide and dexamethasone which produced the conjugate designated as Compound 9 in the present application:



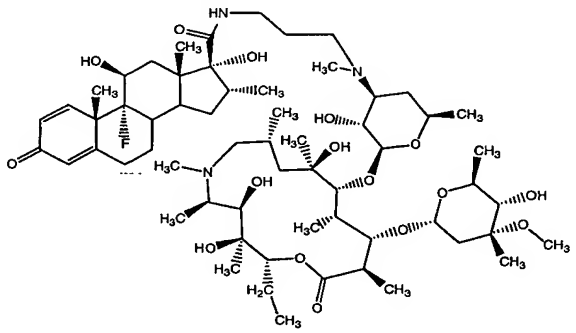
The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. Compound 9 was prepared using the procedure according to the procedure for compound 19, discussed below. This record demonstrates that Compound 9 within the present application was actually reduced to practice prior to February 15, 2002.

(13) To show that, for example, Compound 19 of the present application was synthesized before February 15, 2002, I submit as Exhibit F pages 118-119 from a Laboratory Notebook identified by code OMA-000961-118-A Lab.diary 000961, and an English translation of these pages. Exhibit F sets forth the synthetic protocol for the conjugation of the macrolide and flumethasone which produced the conjugate designated as Compound 19 in the present application:



The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. This record demonstrates that Compound 19 within the present application was actually reduced to practice prior to February 15, 2002.

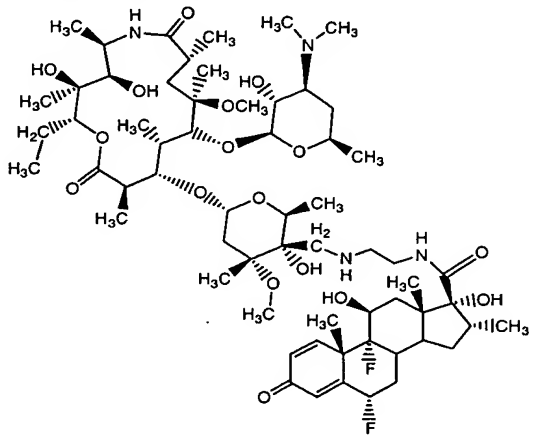
(14) To show that, for example, Compound 23 of the present application was synthesized before February 15, 2002, I submit as Exhibit G page 5 from a Laboratory Notebook identified by code OMA-1127-005-A Lab.diary 1127, and an English translation of this page. Exhibit G sets forth the synthetic protocol for the conjugation of the macrolide and dexamethasone which produced the conjugate designated as Compound 23 in the present application:



The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. This record demonstrates that Compound 23 within the present application was actually reduced to practice prior to February 15, 2002.

(15) To show that, for example, Compound 27 of the present application was synthesized before February 15, 2002, I submit as Exhibit H pages 48 - 49 from a Laboratory

Notebook identified by code VPO-1131- 048-A Lab.diary 1131and an English translation of these pages. Exhibit H sets forth the synthetic protocol for the conjugation of the macrolide and flumethasone which produced the conjugate designated as Compound 27 in the present application:



The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. Compound 27 was prepared by the process provided in pages 20 – 21 of the Laboratory Notebook identified by code VPO-1131- 020-A Lab.diary 1131, submitted as Exhibit I. These records demonstrates that Compound 27 within the present application was actually reduced to practice prior to February 15, 2002.

(16) My laboratory worked diligently on conception and diligent reduction to practice of the claimed invention from a time prior to February 15, 2002, until the filing of provisional application 60/394,670 on July 8, 2002 and thereafter until the filing date of the present application on July 8, 2003. For example, the provisional application 60/394,670 discloses techniques for synthesis of 33 compounds, including Compounds 1, 4, 9, 19, 23, and 27 discussed above, as well as 27 additional compounds that were synthesized prior to filing the provisional application. At least 7 further compounds were synthesized prior to filing the non-provisional application. All of these 40 compounds were synthesized between the conception date (prior to February 15, 2002) and the filing date of the present application. This demonstrates that we worked diligently in order to reduce the claimed compounds to practice.

(17) I declare further that statements made in this Declaration are of my own knowledge and are true and that all statements made on information and belief are believed to be true and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 29.12.2005.

Linda Tomašković

PLIVA d.d.

Istraživački institut

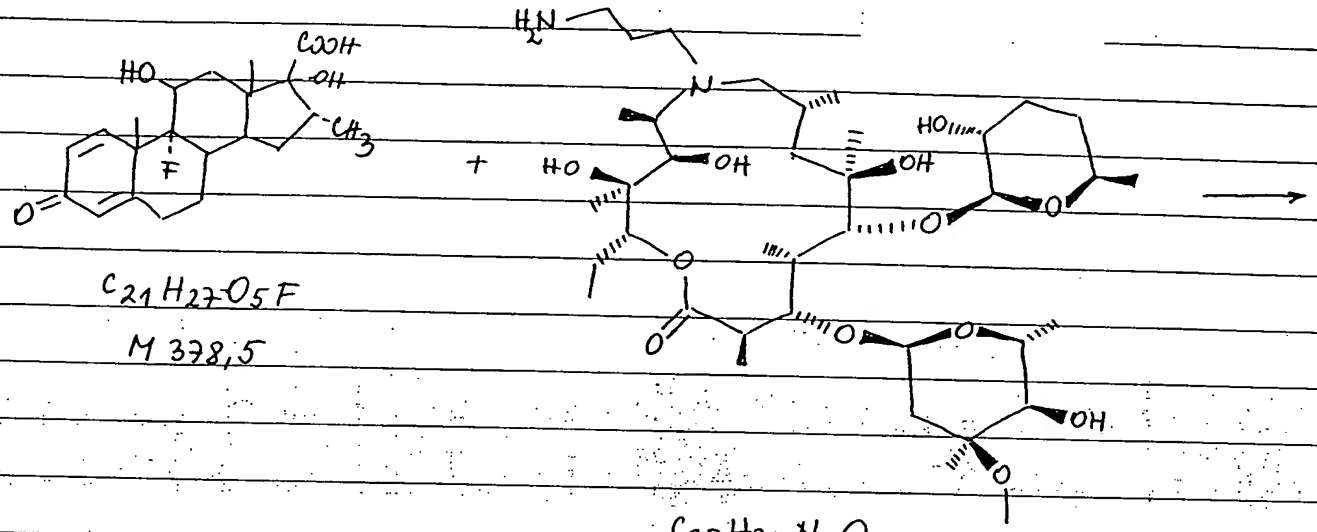
LABORATORIJSKI DNEVNIK

LINDA TOMAŠKOVIĆ № 000959

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: _____ Oznaka LT 77

Datum (i): 30.04.2001.



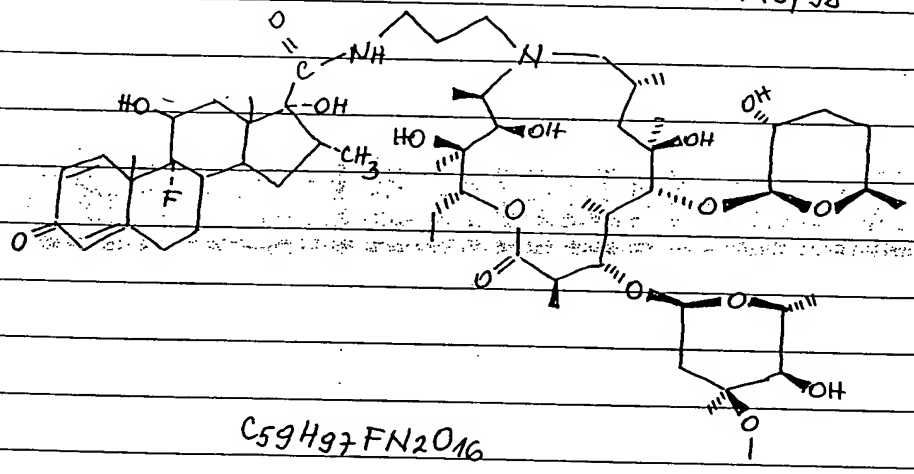
$C_{21}H_{27}O_5F$

M 378,5

+

$C_{38}H_{72}N_2O_{12}$

M 748,98



$C_{59}H_{97}FN_2O_{16}$

M 1109,40

ref: D. Romo et. al., J. Am. Chem. Soc. 120 (1998) 12 237

KEMIKALIJE:

1-hydroxybenzotriazole, ACROS, M 135, 12

1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide · HCl, ALDRICH, M 191,71

Rad obavio: Linda Tomašković Datum: 30.04.2001.

Rad posvjedočio: _____ Datum: 30.4.2001.

Naslov: _____ Oznaka LT 77

Datum (i): 30.04.2001.

Postupak je opisan u dnevniku 0560, str. 106.

Količine reaktanata:

deksametazon-tirozin 76 mg ; 0,2 mmol

TEA 0,253 ml

HOBT 53 mg

amin 150 mg ; 0,2 mmol

EDC · HCl 157 mg

Dobiveno je 0,1666 g čistog produkta. Iskorištenje reakcije 75 %.

MS(ES+) : 1109,7 (MH+)

MS(ES-) : 1107,9 (MH-)

Rad obavio: Linda Tomašković Datum: 30.04.2001.

Rad posvjedočio: _____ Datum: 30.4.2001.

PLIVA d.d.
Research Institute

Title:_____ LabDiary ID LT77
PL14882

Date (i): April 30, 2001

Synthetic scheme: Lab. Diary No. 000959, page 16

Chemicals:
1-hydroxybenzotriazole, ACROS, M 135.12
1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, ALDRICH, M 191.71

Performed by: Linda Tomašković **Date**: April 30, 2001
Witnessed by: Ivana Ozimec **Date**: April 30, 2001

Title:_____ LabDiary ID LT77

Date (i): April 30, 2001

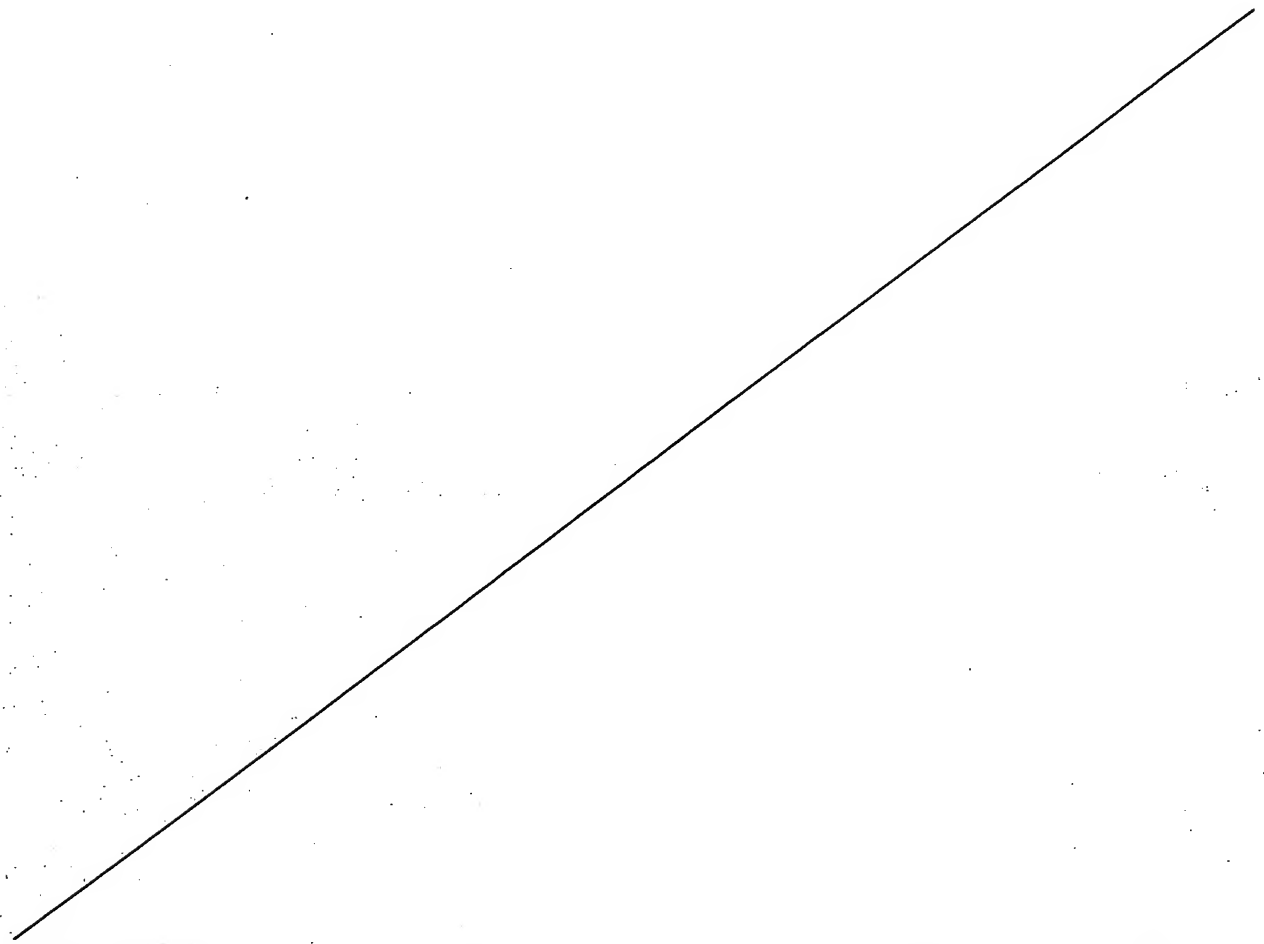
Reaction procedure and data about other chemicals which are used is described in Laboratory Diary Number 0560, page 106.

Amounts:

dexamethasone acid	76 mg; 0.2 mmol
TEA	0.253 ml
HOBT	53 mg
amine	150 mg; 0.2 mmol
EDCxHCl	157 mg

0.1666 g of pure product was obtained. Reaction yield 75%.

$MS(ES^+)$:1109.7 (MH^+)
$MS(ES^-)$:1107.9 (MH^-)



Performed by: Linda Tomašković **Date:** April 30, 2001
Witnessed by: Ivana Ozimec **Date:** April 30, 2001

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

LINDA TOMAŠKOVIĆ

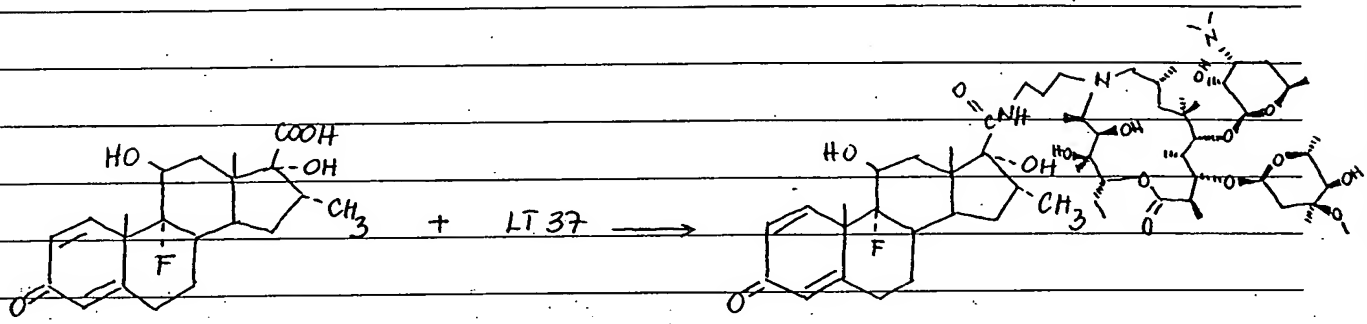
№ 000560

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: _____ Oznaka LT 40

Datum (i): 23. 02 2000

PLR 2146



$C_{21}H_{27}O_5F$ M 378,50 | $C_{40}H_{77}O_{12}N_3$ M 792,4 | $C_{61}H_{102}FN_3O_{16}$ M 1152,47

ref: D. Romo et al., J. Am. Chem. Soc. 120 (1998) 12237

KEMIKALIJE:

1-HYDROXYBENZOTRIAZOLE, ACROS, M 135,12

1-(3-DIMETHYLAMINOPROPYL)-3-ETHYL-CARBODIIMIDE HYDROCHLORIDE, ALDRICH, 28747-069; M 191,71

U suspenziju kiseline LT4 (110 mg; 0,29 mmol) u suhom diklormetanu (5 ml) ohlađenu na 0°C dodano je 0,330 ml trietilamina (otopina iz razbistrila), zatim HOBT (80 mg; 0,59 mmol), amin LT37 (230 mg; 0,29 mmol) i 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (235 mg; 1,2 mmol). Reakcija je provedena na sobnoj temperaturi 24 h uz argon i miješanje.

Rad obavio: Linda Tomašković Datum: 23.02.2000

Rad posvjedočio: [signature] Datum: 13.03.2000

Naslov: _____ Oznaka LT40

Datum (i): 24. 02 2000

Nakon 24 h reakcijska smjesa je uparena na manji volumen na rotavaporu (~2 ml) i očišćena na koloni punjenoj sa silikagelom (eluens: $CHCl_3$: MeOH : NH_4OH = 6 : 1 : 0,1). R_f produkta = 0,57. Dobiveno je 224 mg čistog produkta (iskorištenje reakcije 67%)

MS (ES+) : 1152,8 (MH+)

Rad obavio: Linda Tomašković Datum: 24. 02 2000

Rad posvjedočio: _____ Datum: 13. 03. 2000

Title:_____ LabDiaryID LT40___
PLR 2146

Date (i): February 23, 2000

Synthetic scheme: Lab. Diary No. 000560, page 106

Chemicals:
1-hydroxybenzotriazole, ACROS, M 135.12
1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, ALDRICH, 28747-069; M 191.71

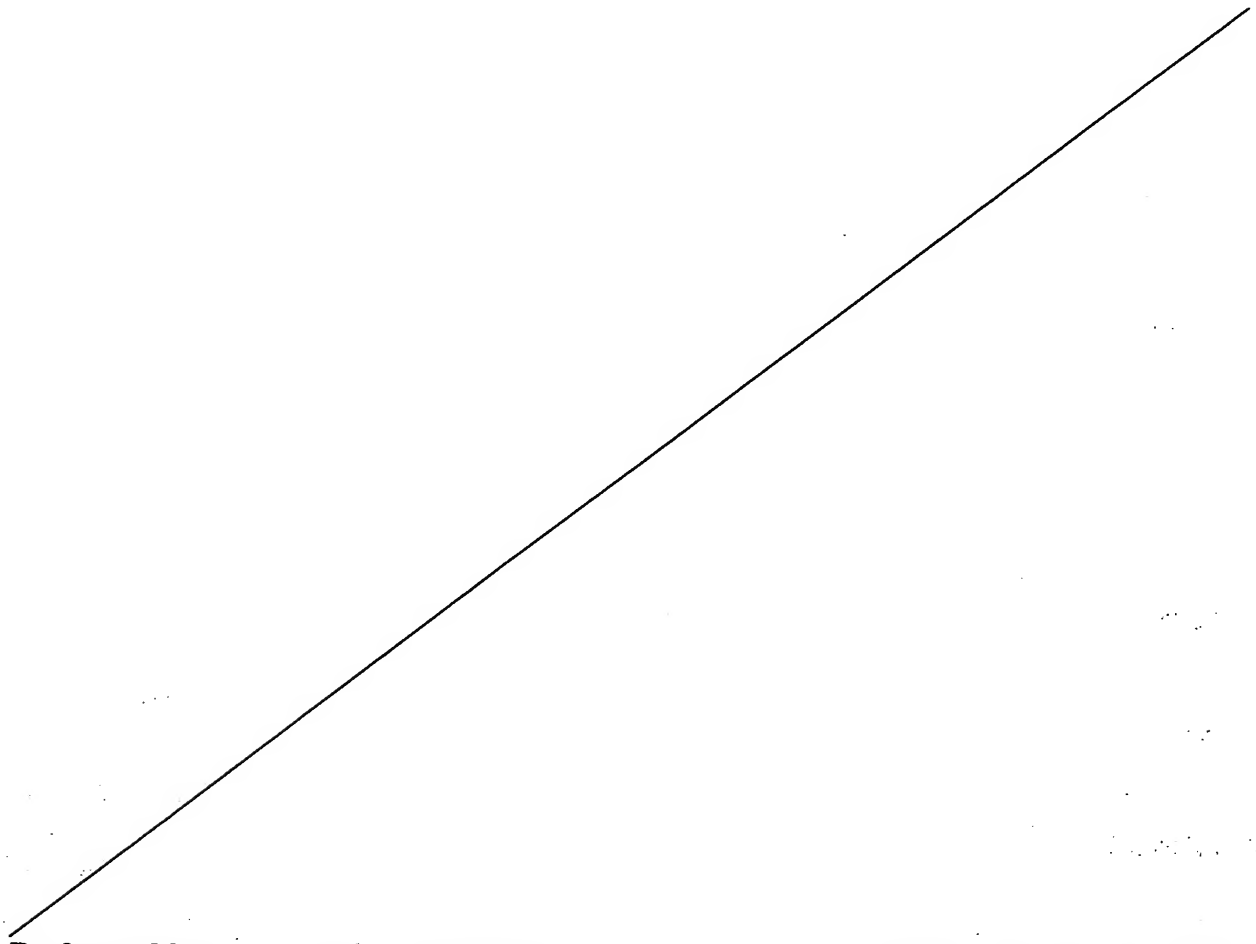
To a suspension of acid LT4 (110 mg; 0.29 mmol) in dry dichloromethane (5 mL) cooled to 0°C under argon, 0.380 mL of triethylamine was added (the solution was cleared), then HOBT (80 mg; 0.59 mmol), amine LT37 (230 mg; 0.29 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (235 mg; 1.2 mmol) were added. The reaction mixture was stirred for 24 hours at room temperature in a flow of argon.

Performed by: Linda Tomašković **Date**: February 23, 2000
Witnessed by: Milan Mesić **Date**: March 13, 2000

Title:_____ LabDiaryID LT40

Date (i): February 24, 2000

After 24 hours the reaction mixture was evaporated to a smaller volume on a rotary evaporator and purified on a silica gel column (eluant: $CHCl_3$: MeOH : NH_4OH = 6 : 1 : 0.1). R_f of the product =0.57. 224 mg of pure product was obtained (yield obtained 67%)
MS(ES^+) :1152.8 (MH^+)



Performed by: Linda Tomašković **Date:** February 24, 2000
Witnessed by: Milan Mesić **Date:** March 13, 2000

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

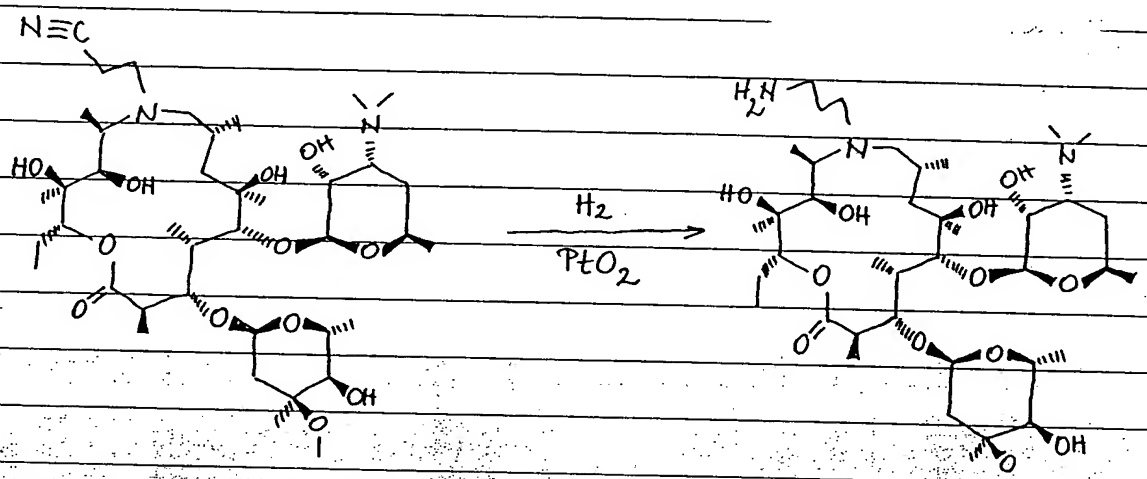
LINDA TOMAŠKOVIĆ

№ 000560

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: _____ Oznaka LT 37

Datum (i): 11. 02. 2000



LT35; $C_{40}H_{73}O_{12}N_3$ (M 788,4) $C_{40}H_{77}O_{12}N_3$ (M 792,4)

ref: D. G. Batt at al., J. Med. Chem. 43 (2000) 41.

KEMIKALIJE:

Platinum (IV) oxide, M 227,09, laboratorij 11

LT 35 (0,7398 g; 0,94 mmol) otopljen je u 40 ml etanola i u to je dodano 130 mg PtO_2. Reakcijska smjesa je stavljena u autoklav i reakcija je provedena uz miješanje i hidriranje pri tlaku od 30 atm 19 h. Nakon toga reakcijska smjesa je profiltrirana i etanol je uparen na rotavaporu. TLC (eluens: $CHCl_3$: MeOH : NH_4OH = 6 : 1 : 0,1) pokazuje smjesu polaznog spoja (R_f = 0,58) i produkta (R_f = 0,13).

Rad obavio: Sinuša Tomašković Datum: 11. 02. 2000

Rad posvjedočio: Mirna Perić Datum: 18. 7. 2000.

Naslov: _____ Oznaka LT 37

Datum (i): 12.02.2000

Reakcijska smjesa je očišćena na koloni punjenoj sa silikagelom (eluens: CH_2Cl_2 : MeOH : NH_4OH = 30 : 50 : 2)
Dobiveno je 108 mg čistog produkta. Žuto ulje
Reakcija 15 %. MS (ES+): 792,6 (MH^+)

Rad obavio: Linda Fumagalli Datum: 12.02.2000

Rad posvjedočio: Ljiljana Pilić Datum: 14.2.2000.

Title:_____ LabDiaryID LT37
PLR 2150

Date (i): February 11, 2000

Synthetic scheme: Lab. Diary No. 000560, page 98

Chemicals:
Platinum(IV)oxide, M 227.09, laboratory 11

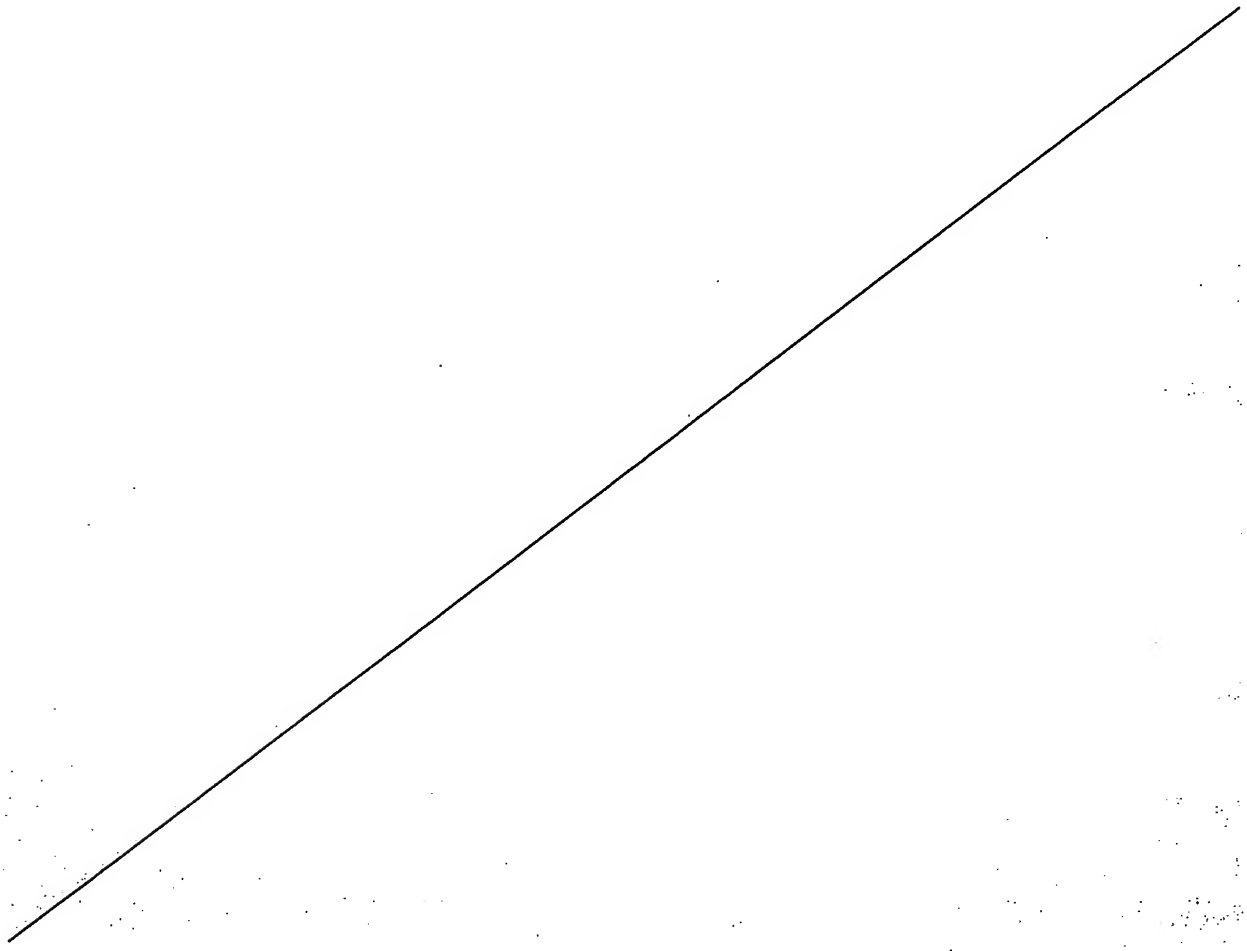
LT35 (0.7398 g; 0.94 mmol) was dissolved in 40 ml ethanol and 130 mg PtO_2 was added. The reaction was performed in an autoclave during 19 hours under the pressure of 30 atm. Subsequently, the reaction mixture was filtered and ethanol was evaporated on a rotary evaporator. TLC (eluant: $CHCl_3$: methanol : NH_4OH = 6 : 1 : 0.1) showed mixture of starting compound (R_f= 0.58) and product (R_f=0.13).

Performed by: Linda Tomašković **Date:** February 11, 2000
Witnessed by: Dijana Pešić **Date:** February 18, 2000

Title:_____ LabDiaryID LT37

Date (i): February 12, 2000

Reaction mixture was purified on a silica gel column (eluant: CH_2Cl_2 : MeOH : NH_4OH = 30 : 50 : 2). 108 mg of pure product was obtained. Yield obtained 15%. MS(ES^+) :792.6 (MH^+)



Performed by: Linda Tomašković **Date:** February 12, 2000
Witnessed by: Dijana Pešić **Date:** February 18, 2000

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

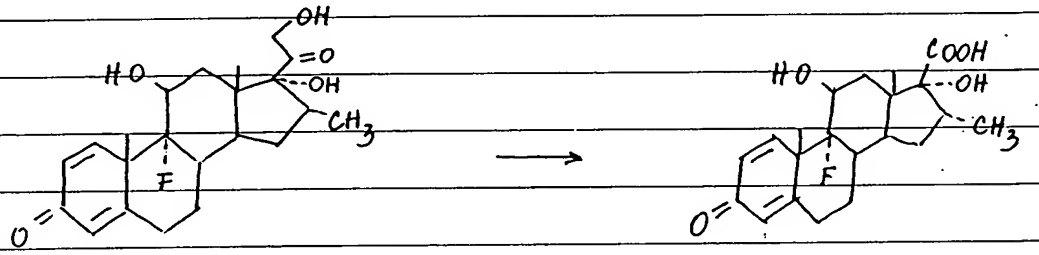
LINDA TOMAŠKOVIĆ

№ 000560

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: _____ Oznaka LTH

Datum (i): 20.04 '99.



M 392,47 M 378,5

ref: K. Meyer and T. Reichstein, Helv. Chim. Acta 30(1947) 1508

KEMIKALIJE

DEKSAMETAZON - PLIVA, šifra standarda 0033, siniji liq 04

HJO_4 - KEMIKA M 192

DIOKSAN, p.a. - KEMIKA S: 9-16-33

U okrugloj tikvici od 100 ml otopljeno je 1 g (2,5 mmol) deksametazona u 50 ml dioksana u otopinu je zatim dodano 1,44 g (7,5 mmol) perjodatne kiseline otopljene u 20 ml H_2O. Reakcija je provedena na sobnoj temperaturi 3 h. Dioksan je uparen na rotavapotu, a ostatak je zakiseljen s razrijeđenom H_2SO_4. Bijeli kristalinični talog je odfiltriran preko Büchnera.

Dobiveno je 0,8642 g produkta (iskorištenje reakcije 90%)

Rad obavio: Ljiljana Tomašević Datum: 20.04 '99.

Rad posvjedočio: Mirjana Paret Datum: 5-6. 1999.

PLIVA d.d.
Research Institute

Title:_____ LabDiaryID LT4

Date (i): April 20, 1999

Synthetic scheme: Lab. Diary No. 000560, page 59

Chemicals:
Dexamethasone- PLIVA, standard code 0033, serial number 04
HIO_4- KEMIKA M192
Dioxane, p.a.- KEMIKA s:9-16-33

Dexamethasone (1g; 2.5 mmol) was dissolved in 50 ml dioxane in a 100-ml round-bottomed flask. To this solution was added periodic acid (1.44 g; 7.5 mmol) dissolved in 20 ml H_2O. The reaction was performed at the room temperature for 3 hours. Dioxane was then evaporated on a rotary evaporator and the residue was acidified with diluted H_2SO_4. The white precipitate was filtered off by using Buchner funnel.
0.8642 g of product was obtained. Reaction yield obtained 90% .

Performed by: Linda Tomašković **Date:** April 20, 1999
Witnessed by: Dijana Parat **Date:** June 06, 1999

PLIVA d.d.

Istraživanje i razvoj

LABORATORIJSKI DNEVNIK

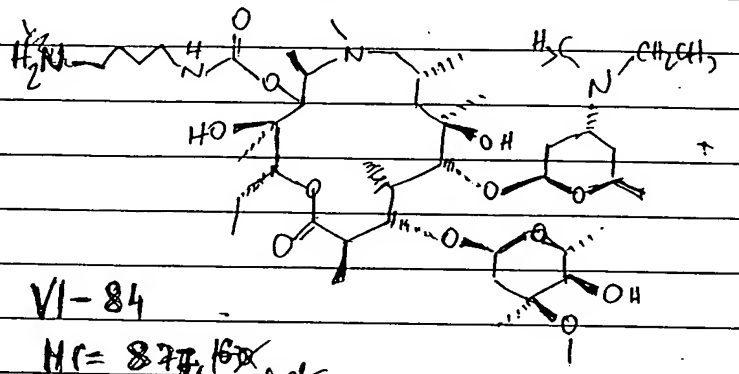
VIŠNJA POLJAK

1131

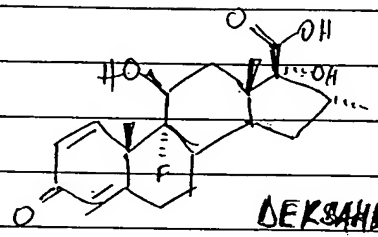
Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: _____ Oznaka VI-85
PL 22714

Datum (i): 29. 1. 2002

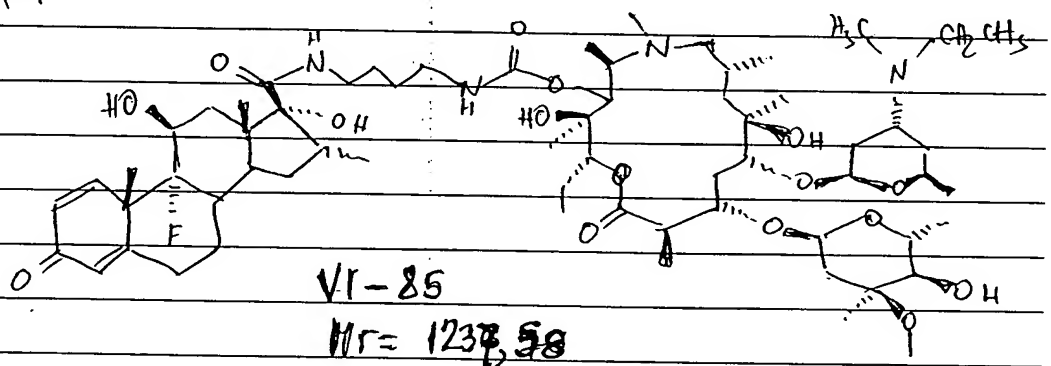


VI-84
Mr = ~~877,60~~ 876,60
pdpd



DEKSAMETAZON KISELINA
Mr = 378,16

HOBT, TEA
EDC·HCl



VI-85
Mr = ~~1237,58~~ 1237,58
pdpd

Reakcija:

POLAZNI MATERIJAL:

VI-84, Mr = 876,60
DEKSAMETAZON KISELINA, Mr = 378,16
TRIETILAMIN, IZNAD KOH
HIDROKSIBENZOTRIAZOL, ACROS lot-Nr: ~~A0125 87801~~
EDC HCl, osušen iz sobe 6
DIKLORMETAN, osušen nad molekulskim sitom 0,3 nm

Rad obavio: Vitja Paljak Datum: 29. 1. 2002.

Rad posvjedočio: Linda Tamašić Datum: 29.01.2002.

PLIVA d.d.
Istraživanje i razvoj

Naslov: ________________ Oznaka VI-85

Datum (i): 29.1.2002.

POSTUPAK DOBIVANJA:

U tikvici propuhavoj argonom otopljeno je 425,0 mg (0,3332 mmol) dekosameftazol kiseline u 15 ml diklormetana (suhog).
U reakcijsku smjesu dodano je zatim 0,3632 ml (7,818 eq) tri-etilamina, pri čemu se otopina razbistri. Dodano je zatim 0,0900 g (2 eq) hidroksibenzotriazola, te brzo 292,1 mg (0,3332 mmol) spoja VI-84. U otopinu je na kraju dodano još 4 eq odnosno 0,2292 g EDC·HCl. Reakcijska smjesa je miješana na sobnoj temperaturi 24 h. Otapalo je upareno a spoj očišćen na koloni u sustavu otapala $CHCl_3$: MeOH : NH_4OH = 6:1:0,1
2. puta spoj je očišćen u sustavu $CHCl_3$: MeOH : NH_4OH = 90:8:1.

DOBIVENO PRODUKTA:

m(VI-85, čist) = 0,0578 g

Pločice priložene u bilježnici

MS(MH+) = 1237,82
vidjeti u prilogu.

Rad obavio: Višnja Poljak Datum: 29.1.2002.

Rad posvjedočio: Linda Tomašković Datum: 29.01.2002.

PLIVA d.d.
Research Institute

Title:_____ Lab diary ID VI-85
PL 27714

Date (i): 29.1. 2002.

Scheme in lab notebook No. 1131

Reference:

STARTING MATERIAL:

VI 84, Mr=876,60
Dexamethasone acid, Mr=378,16
triethylamine, over KOH
Hydroxybenzotriazole, Acros, Lot.NO. AO12582601
EDCxHCl, borrowed from the room 6
dichloromethane, dried over molecular sieves 0,3 nm

Performed by: Visnja Poljak **Date:** 29.1. 2002.
Witnessed by: Linda Tomaskovic **Date:** 29.1. 2002.

PLIVA d.d.
Research Institute

Title:_____ Lab diary ID VI 85

Date (i): 29.1. 2002.

PREPARATION METHOD:

In a flask blown up with argon, 126,0 mg (0,3332 mg) of dexamethasone acid was disolved in 15 ml of (dry) dichloromethane.
In the reaction mixture 0,3632 ml (7,818 eq) of triethylamine was then added, and the mixure cleared.
0,0900 g (2eq) of hydroxybenzotriazole was added, and then quickly 292,1 mg (0,3332 mmol) of compound VI-84. In the mixture was in the end added 4 eq i.e. 0,2292 g EDCxHCl. The reaction mixture was stirred at room temperature for 24 h. Solvent was then evaporated, and the product purified on a column in the solvent system CH_3:MeOH:NH_4OH=90:8:1.

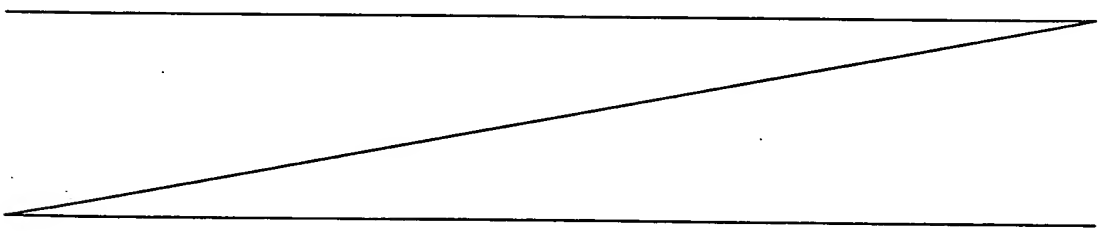
PRODUCT OBTAINED:

m(VI-85)= 0,0578 g

TLC plates in the notebook.

MS(MH+)=1237,82

Spectra in attachement.



Performed by: Visnja Poljak **Date:** 29.1. 2002.
Witnessed by: Linda Tomaskovic **Date:** 29.1. 2002.

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

№ 000961

ORESTA MAKARUNA

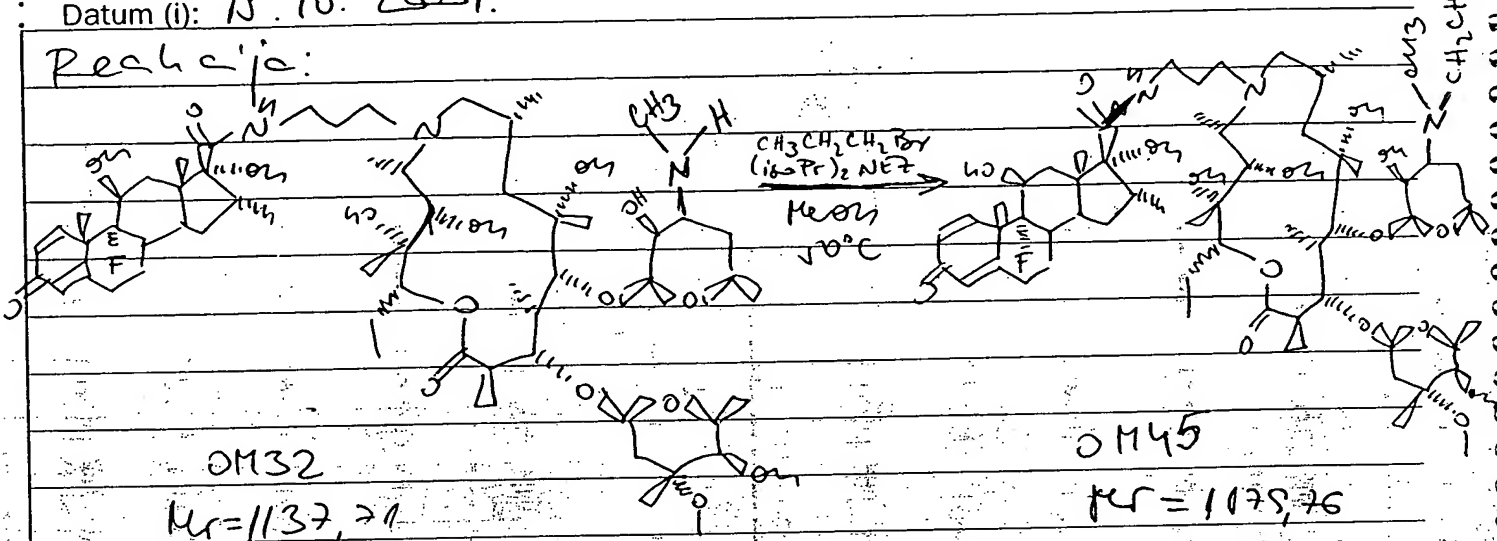
DNEVNIK 2

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: _____ Oznaka OM45-1

Datum (i): 15.10.2001.

Reakcija:



OM32
Mr=1137,71
$C_{60}H_{100}FN_3O_{16}$

OM45
Mr=1175,76
$C_{63}H_{106}FN_3O_{16}$

Uvenila je:

OM32 m=90 mg n=0,079 mmol ρ=0,76 g/cm³
(iPr)₂NEt n=0,6717 mmol V=115 µl Mr=129,25
$CH_3CH_2CH_2Br$ n=0,1011 mmol Mr=123 ρ=1,35 g/ml V=457 µl
MeOH V=2 ml

Reference i postupak opisani su u str. 118/119.

Čišćenje je u ovom slučaju provedeno sustavom eluensa CH_2Cl_2 : MeOH : NH_4OH = 6 : 1 : 0,1

m(OM45) = 20 mg
m(OM45 nešto zaostanuje) = 26 mg

MS (MH+) 1180,59

Rad obavio: _____ Datum: 24.10.2001.

Rad posvjedočio: _____ Datum: 24.10.2001.

Title:_____ LabDiaryID OM45-1

Date (i): October 15, 2001

Synthetic scheme: Lab. Diary No. 000961, page 124

Chemicals:
OM32 m=90 mg n=0.079 mmol ρ=0.76 g/cm^3
$(isoPr)_2NEt$ n=0.6717 mmol V=115µL M 129.25
$CH_3CH_2CH_2Br$ n=0.5011 mmol M 123 ρ=1.35 g/mL V=45.7 µL
MeOH - V=2 mL

Reference and procedure are described on pages 118/119.

The reaction mixture was purified on a silica gel column with eluant: $CHCl_3$: MeOH : NH_4OH = 6 : 1 : 0.1.

m(OM45)=20 mg
m(OM45 unpure)=26 mg

MS(MH^*): 1180.59

Performed by: Oresta Makaruha **Date**: October 24, 2001
Witnessed by: Linda Tomašković **Date**: October 24, 2001

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

№ 000961

ORESTA MAKARUNA

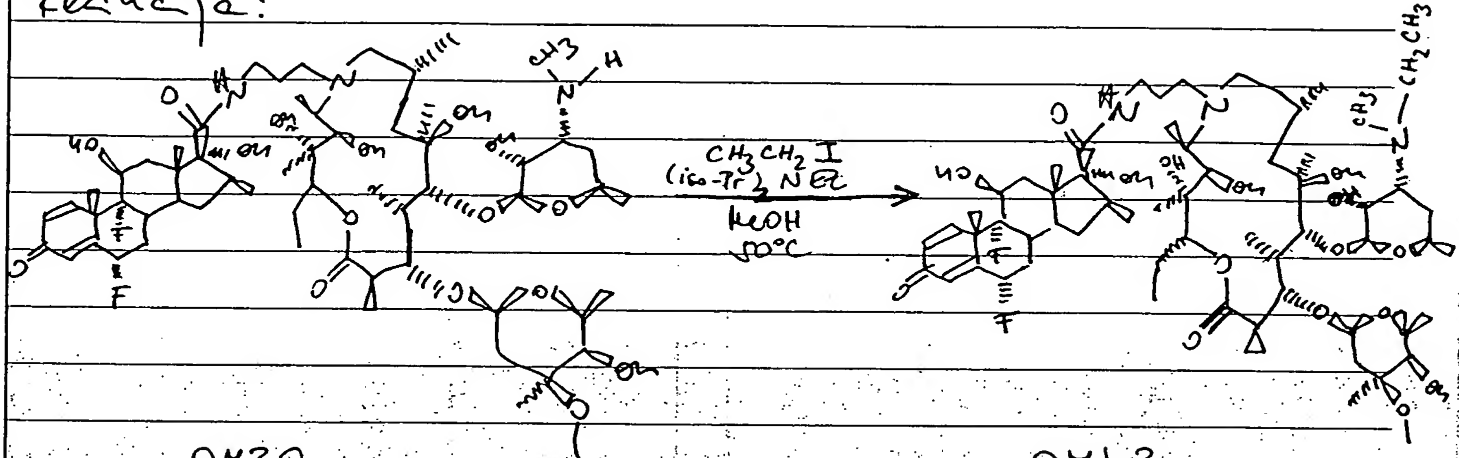
DNEVNIK 2

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: ______ Oznaka OM43-1

Datum (i): 25.09.2001.

Reakcija:



OM39
Mr = 1155,70
$C_{60}H_{99}F_2N_3O_{16}$

OM43
Mr = 1183,75
$C_{62}H_{103}F_2N_3O_{16}$

Ref. K. Tanizaki, T. Sunazuka, S. Mani, H. Toyoda, S. Ōmura, N. Inatomi, Z. Itoh ; Chem. Pharm. Bull. 37 (10) 2687–2700 (1989)

Kemikalije:
OM39 (Mr = 1155,70) n(OM39) = 0,0588 mmol
N,N-diisopropylethylamine, Merck, S25410 844 ; ρ = 0,76 Mr = 129,25 n(iso-Pr)2NEt) = 0,5 mmol
1-jodoethane pomaknut iz ??e 6. ρ = 1,910–1,940 Mr = 155,97 n(CH3CH2I) = 0,373
MeOH, Kemika

Postupak:
U tikvici od 10 ml otopljeno je 68 mg OM39 u 2 ml MeOH. Otopini je dodano 85.6 μl N,N-diisopropylethylamine i 112,5 μl iodoethane. Miješano je na magnetskoj mješalici 20 sati na temperaturi od 50 °C. Reakcijska smjesa

Rad obavio: [signature] Datum: 26.09.2001.

Rad posvjedočio: Jinoke Tomašković Datum: 2.10.2001.

Naslov:

Oznaka: OM43-1

Datum (i): 25.09.2001.

Razrijeđeno je s 20 ml EtOAc i isprano s 20 ml $NaHCO_3$ (zasić.) i 20 ml H_2O. Organska faza sušena je na bezvodnom Na_2SO_4. Otapalo je upareno na rotavaporu.

Smjesa produkta i polaznog spoja očišćena je na koloni punjenoj silikagelom u sistemu $CHCl_3:MeOH:NH_4OH = 6:1:0.1$.

m(OM43) = 38 mg iskorištenje 35%
m(OM39 neizreagiranog) = 24 mg

MS (MH^+) 1185.7

Rad obavio: [illegible signature] Datum: 26.09.2001.

Rad posvjedočio: [illegible signature] Datum: 2.10.2001.

Title:_____ LabDiary ID OM43-1

Date (i): September 25, 2001

Reaction:

Synthetic scheme: Lab. Diary No. 000961, page 118

REF. : K. Tsuzuki, T. Sunazuka, S. Marni, H. Toyock, S. Omura, N. Inatomi, Z. Itok; Chem. Pharm. Bull. 37 (10) 2687-2700 (1989)

Chemicals:
OM39 (Mr=1155,70) n(OM39)=0,0588 mmol
N,N-diisopropylethylamine, Merck, S25410 844, δ=0,76 kg/l; Mr=129,25; n((iso-Pr)$_2$NEt)=0,5 mmol
1-iodoethane borrowed from room 6. δ=1,910-1,940 Mr=155,97 n(CH_3CH_2I)=0,373
MeOH, Kemika

Procedure:
In flask (10 ml) was dissolved 68 mg of compound OM 39 in 2 ml of MeOH. In the solution were added 85,6 µl of N,N-diisopropylethylamine and 112,5 µl of iodoethane. The reaction mixture was stirred at 50 °C for 20 hours. The reaction mixture

Performed by: Oresta Makaruha **Date**: September 26, 2001
Witnessed by: Linda Tomašković **Date**: October 02, 2001

Title:_____ LabDiary ID OM43-1

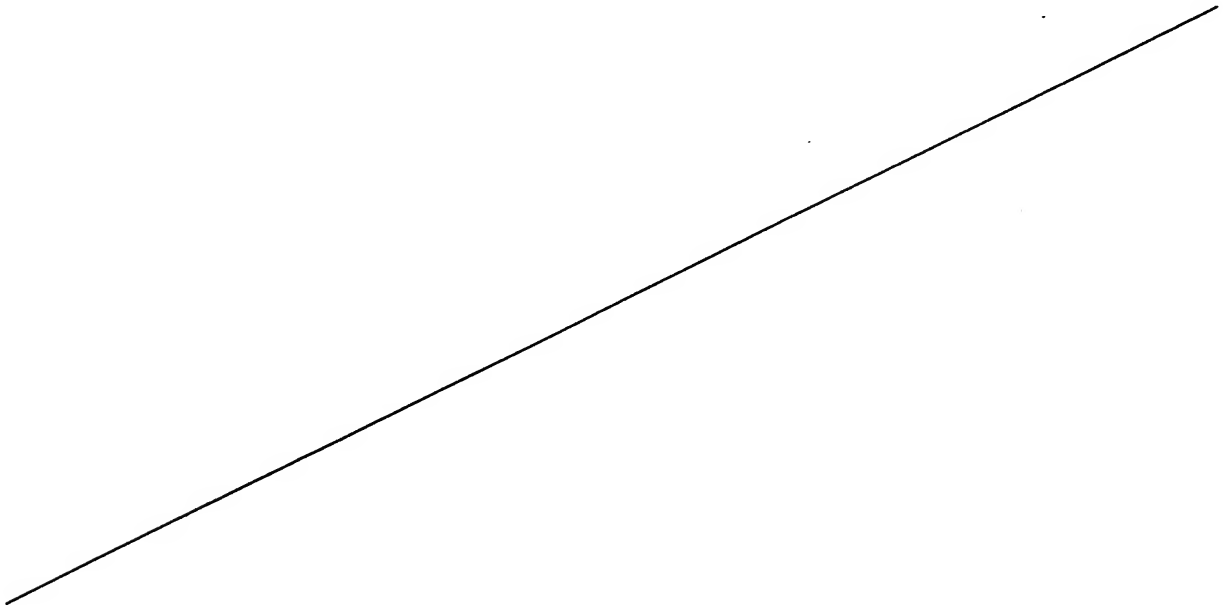
Date (i): September 25, 2001

was diluted with 20 ml of EtOAc and washed with 20 ml of $NaHCO_3$ (satur.) and 20 ml of H_2O. Organic layer was dried over anhydrous Na_2SO_4. Solvent was evaporated under vacuum.

The mixture of product and initial compound was purified on a silica gel column in the solvent system CH_2Cl_2:MeOH:NH_4OH 6:1:0,1.

m (OM43)=89,1 mg purity 95%
m (OM39, initial compound)=24 mg

MS (MH^+) 1185,7



Performed by: Oresta Makaruha **Date**: September 26, 2001
Witnessed by: Linda Tomašković **Date**: October 02, 2001

PLIVA d.d.

Istraživanje i razvoj

LABORATORIJSKI DNEVNIK

ORESTA MAKARUNA

DNEVNIK 3

1127

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

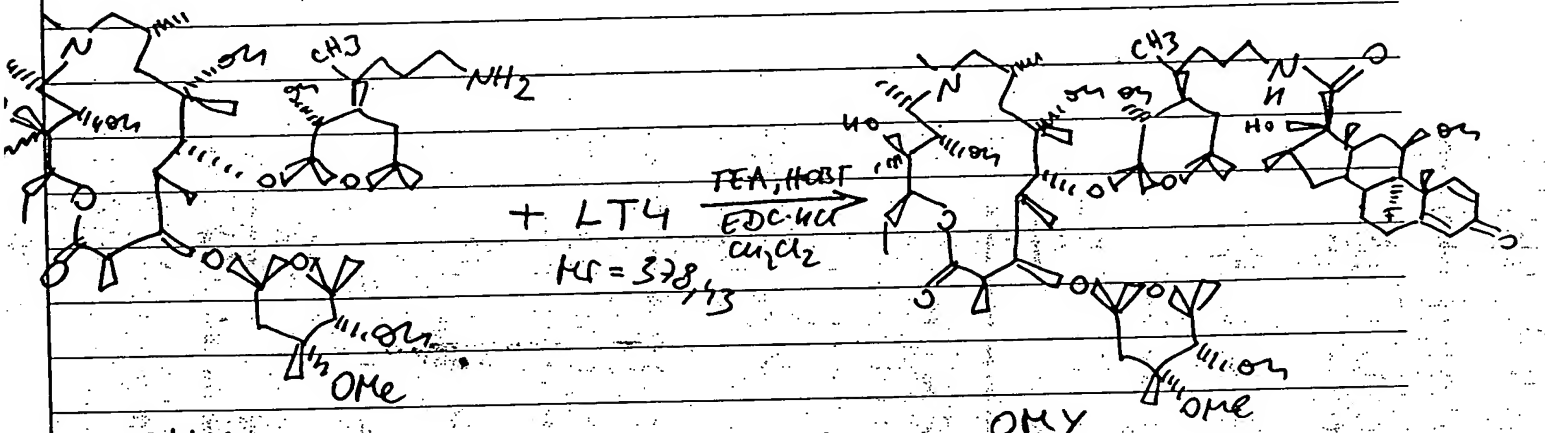
PLIVA dd
Istraživanje i razvoj

Naslov: _____ Oznaka ~~OMY-2~~ OMY-1

PL 27439

Datum (i): 19. 11. 2001.

Reakcija:



OMX
Mr = 791,55

OMY
Mr = 1151,72

Reference i postupak opisani u ~ HW. 56/57 u laboratorijskom dnevniku br. 917 !!

Utrošci:

OMX Mr = 791,55 n = 0,221 mmol m = 175 mg
CH_2Cl_2 V = 5 mL
LT4 (dobiven od L. Tomašković, lab. dn. br. 506, str. 98) n = 0,221 mmol m = 83,7 mg
HOBT ~~m = 23 mg~~ 61 mg
EDC·HCl ~~m = 179,22 mg~~
TEA V = 290 µL

Produkt je dobiven u kolonici punjenoj silikagelom uz eluens CH_2Cl_2 : MeOH : NH_4OH = 6 : 1 : 0,1.

m(OMY) = 15,8 mg 95,17% pura UV MS(MH+) 1152,53

Rad obavio: _____ Datum: 20. 11. 2001.

Rad posvjedočio: Ljubica Tomašković Datum: 27. 11. 2001.

Title: _____ LabDiary ID OMY-1
PL27439

Date (i): November 19, 2001

Reaction:

Synthetic scheme: Lab. Diary No. 001127, page 5

Reference and reaction procedure are described in Laboratory Diary Number 0917, pages 56/57

Chemicals:
OMX Mr=791,55 n=0,221 mmol m=175 mg
CH_2Cl_2 5 ml
LT4 (received from Linda Tomašković, Lab. Diary No. 000506, page 98) n=0,221 mmol m=83,7 mg
HOBT m=61 mg
EDCxHCl m=179,22 mg
TEA V=290 µl

Product was purified on a silica gel column in the solvent system CH_2Cl_2:MeOH:NH_4OH 6:1:0,1.

m(OMY)=15,8 mg 95,17% according to UV MS(MH^+) 1152,53

Performed by: Oresta Makaruha **Date:** November 20, 2000
Witnessed by: Linda Tomašković **Date:** November 27, 2000

PLIVA d.d.

Istraživanje i razvoj

LABORATORIJSKI DNEVNIK

VIŠNJA POLJAK

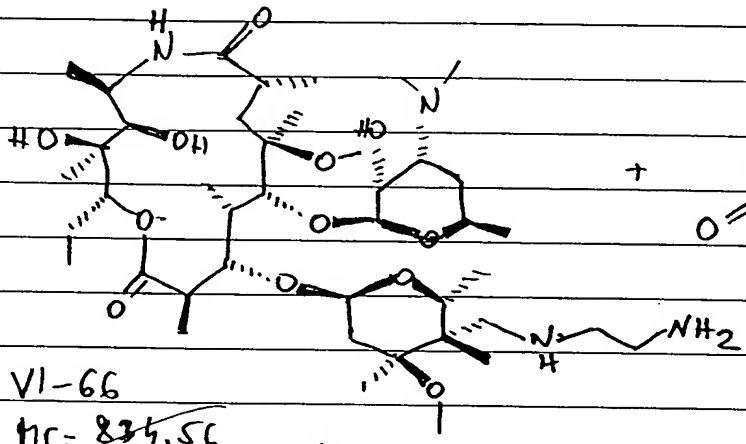
1131

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: _____ Oznaka VI-70
PL 27617

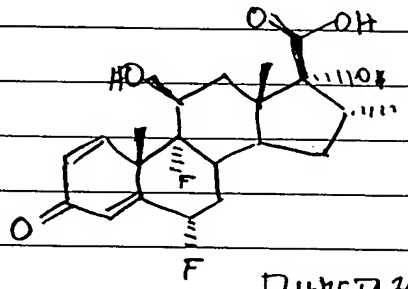
Datum (i): 17.12.2001.

REAKCIJA:



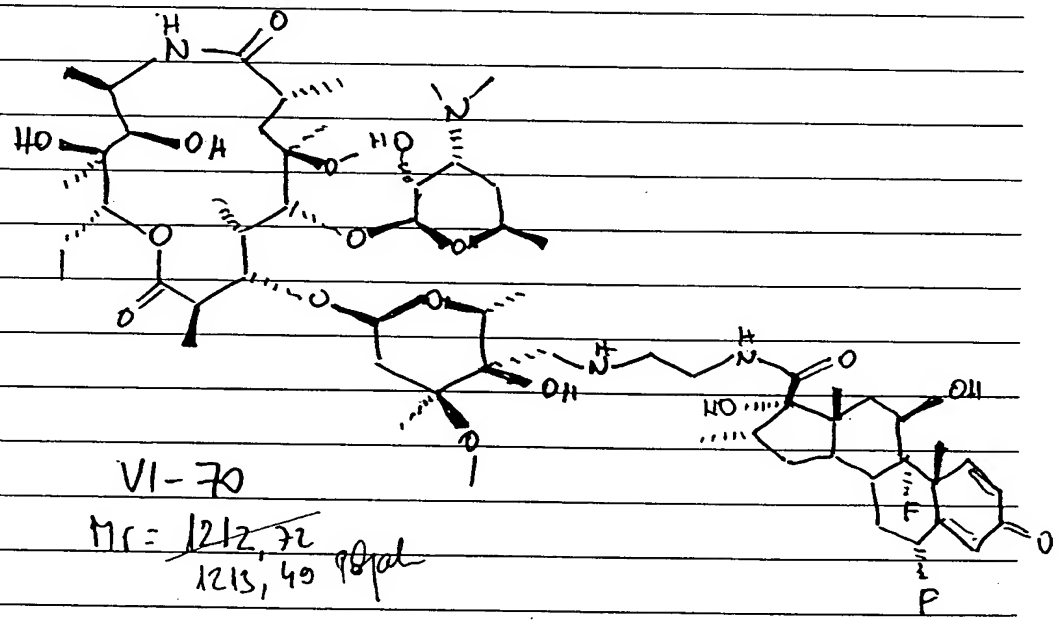
VI-66
Mr = ~~834,51~~ 835,08 g/mol

+



FLUMETAZON KISELINA
Mr = 396,17

TEA
HOBT, EDC·HCl



VI-70
Mr = ~~1212,72~~ 1213,40 g/mol

REFERENCA:

Rad obavio: Višnja Rajsl Datum: 17.12.2001.

Rad posvjedočio: Linda Trinajstić Datum: 19.12.2001.

Naslov: _____ Oznaka VI-70

Datum (i): 17.12. 2001.

POLAZNI MATERIJAL:

VI-66 Mr = 834,56 m = 0,1411 g n = 0,1178 mmol
DIKLORMETAN, osušen iznad molekulskog sita 0,4 nm V = 6,5 ml
FLUHETAZON KISELINA, m = 46,68 mg n = 0,1178 mmol
TRIETILAMIN, osušen iznad KOH V = 0,128 ml n = 0,9209 mmol
HIDROKSIBENZOTRIAZOL, ACROS m = 0,03175 g n = 0,2356 mmol
EDC·HCl m = 0,4693 g n = 0,4412 mmol

POSTUPAK DOBIVANJA:

postupak dobivanja kao za VI-54 na str. 20

DOBIVENO PRODUKTA:

Nakon ošćenja na koloni punjenoj silikagelom u sustavu otapala $CHCl_3$: MeOH : NH_4OH = 6 : 1 : 0,1

m (VI-70) = 0,082 g

$MS\left(\frac{M}{2}H^+\right)$ = 607,44 spektar u prilogu

Rad obavio: Vesna Polzak Datum: 17.12 2001.

Rad posvjedočio: Linda Tamarut Datum: 19.12. 2001.

PLIVA d.d.
Research Institute

Title:_____ Lab diary ID VI-70
PL 27617

Date (i): 17.12.2001.

REACTION SCHEME:

Scheme in lab notebook No. 1131

Reference:

Performed by: Visnja Poljak **Date**: 17.12.2001.
Witnessed by: Linda Tomaskovic **Date**: 19.12. 2001.

Title:_____ Lab diary ID VI-70

Date (i): 17.12. 2001.

STARTING MATERIAL:

VI-66 Mr=834,56 m=0,1411 g n=0,1178 mmol
Dichloromethane, dried over molecular sieves 0,4 nm V=6,5 ml
Flumethasone acid, m=46,68 mg, n=0,1178 mmol
Triethylamine, dried over KOH V=0,128 ml n=0,9209 mmol
Hydroxybenzotriazole, Acros m=0,03175 g n=0,2356 mmol
EDCxHC m=0,1493 n=0,4712 mmol

PREPARATION METHOD:

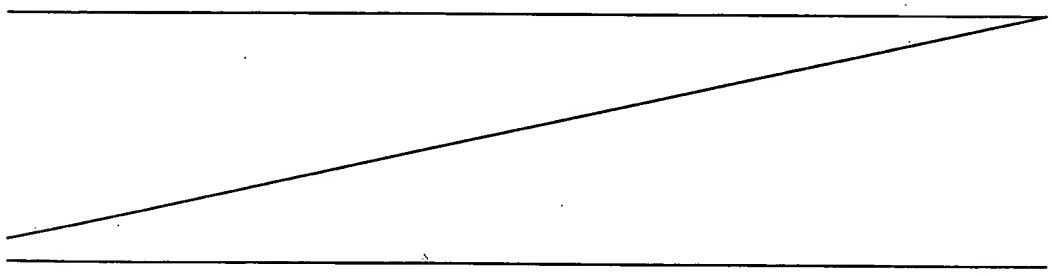
Preparation method as for VI 54 on page 20.

PRODUCT OBTAINED:

After purification on a silicagel column in the solvent system CH_3Cl:MeOH:NH_4OH=6:1:0,1.

m(VI-70)= 0,082 g HPLC-MS spectra are atteched.

MS(MH^+)/2=607,44 spectras attached



Performed by: Visnja Poljak **Date:** 17.12. 2001.
Witnessed by: Linda Tomaskovic **Date:** 19.12.2001.

PLIVA d.d.

Istraživanje i razvoj

LABORATORIJSKI DNEVNIK

VIŠNJA POLJAK

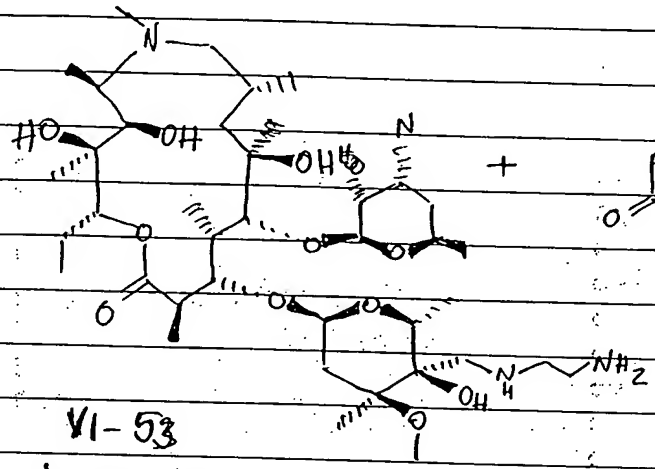
1131

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

Naslov: _____ Oznaka VI-54
PL 27468

Datum (i): 27. 11. 2001.

REAKCIJA:



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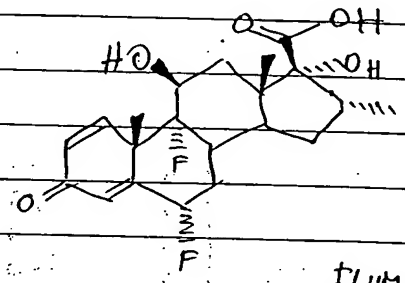
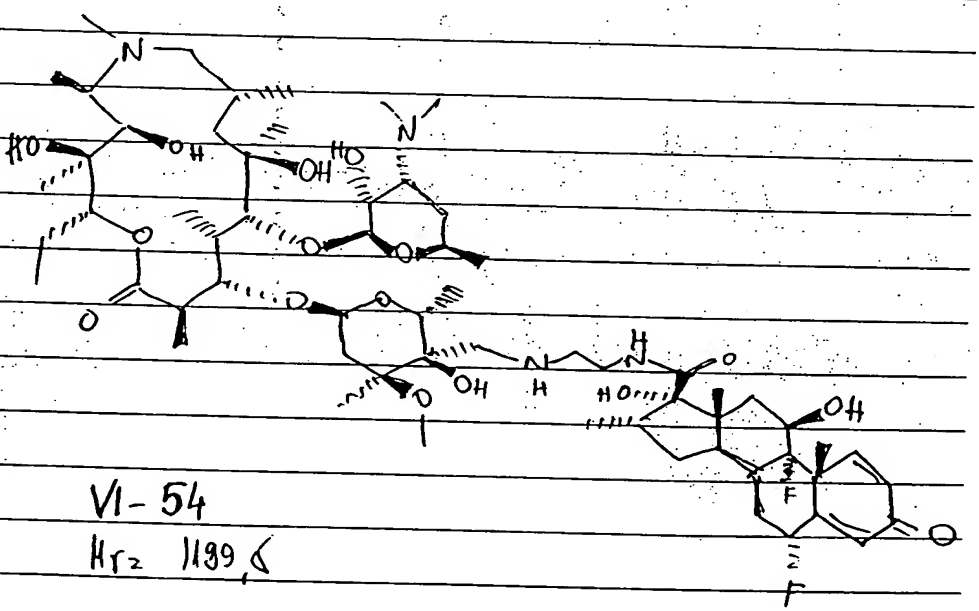


FLUMETAZON KISELINA
Mr = 396,42

VI-53
Mr = 820,58

TEA
HOBT, ~~EDC HCl~~



VI-54
Mr = 1199,6

Reference:

Rad obavio: Ivica Poljak Datum: 27.11.2001.

Rad posvjedočio: Linda Tomašić Datum: 29-11-2001.

Naslov: _____ Oznaka VI-54

Datum (i): 27.11.2001.

POLAZNI MATERIJAL:

VI-53 (Mr = 820,58)
FLUMETAZON KISELINA Mr = 396,42
TRIETILAMIN, OSUŠEN IZNAD KOH
HIDROKSIBENZOTRIAZOL, ACROS, lot.Nr: A0125826 01
EDC HCl
DIKLORMETAN, osušen nad molekulskim sitima 0,4 nm

POSTUPAK DOBIVANJA:

U 10 ml diklormetana kroz koji je propuhan argon dodano je 0,1163 g (0,2933 mmol) flumetazonkiselina. U reakcijsku smjesu dodano je zatim 0,320 ml TEA (2,293 mmol) pri čemu se otopina razbistri. Dodano je zatim 0,0793 g HOBT ; zatim brzo 0,2408 g VI-53 (0,2933 mmol), te 0,2017 g (1,1732 mmol) EDC·HCl.
Reakcijska smjesa je miješana 24 h na sobnoj temperaturi pod argonom. Otapalo je zatim upareno, a sporo čišćenje na koloni sa sustavom otapala $CHCl_3$: MeOH : NH_4OH = 6 : 1 : 0,1. Još je bilo potrebno ostatak još jednom (u istom sustavu) čistiti.

DOBIVENO PRODUKTA:

m(VI-54) = 0,2141 g.

Spektri HPLC-MS priloženi. MS(MH+) = 1093,0

Rad obavio: Višnja Pejaković Datum: 27.11.2001.

Rad posvjedočio: Linda Tamašković Datum: 29.11.2001.

PLIVA d.d.
Research Institute

Title:_____

Lab diary ID VI-54
PL 27468

Date (i): 27.11.2001.

REACTION SCHEME:

Scheme in lab notebook No. 1131

Reference:

Performed by: Visnja Poljak **Date:** 27.11. 2001.
Witnessed by: Linda Tomaskovic **Date:** 29.11. 2001.

PLIVA d.d.
Research Institute

Title:_____ Lab diary ID VI-54

Date (i): 27.11. 2001.

STARTING MATERIAL:

VI-53 (Mr=820,58)
Flumethasone acid, Mr=396,42
Triethylamine, dried over KOH
Hydroxybenzotriazole, Acros, Lot.No. AO12582601
EDCxHC
Dichloromethane, dried over molecular sieves 0,4 nm

PREPARATION METHOD:

To 10 ml diclorometh ane blown up with argon 0,1163 g (0,2933 mmol) of flumethasone acid was added. To the reaction mixture 0,320 ml TEA (2,293 mmol) was then added and the mixture cleared. 0,0793 g (10,5866 mmol) HOBT was then added and then quickly 0,2408 g VI-53 (0,2933) and 0,2017 g (1,1732 mmol) EDCxHCl.
Reaction mixture was stirred for 24 h at room temperature under argon. Solvent was then evaporated, and the compound purified on a column in the solvent system CH_3Cl:MeOH:NH_4OH=6:1:0,1
The compound had to be purified once again (in the same system).

PRODUCT OBTAINED:

m(VI-54)= 0,2141 g

HPLC-MS spectra are atteched. MS(MH+)=1200,0

Performed by: Visnja Poljak **Date:** 27.11. 2001.
Witnessed by: Linda Tomaskovic **Date:** 29.11.2001.